LEVEL III

(13)

A024989

Office of Naval Research

Contract No. N00014-75-C-1086, TR#6
• N00014-75-C-0287

Task No. NR-105-539

Technical Report No. 7 no. 7 (Annual), 1 May
78-30 Apr 79,

Treatment of Hemorrhagic Shock
versus
Saline vs Colloid.

By

Gerald S. Moss M. D.
Chairman, Department of Surgery
Michael Reese Hospital and Medical Center
2929 South Ellis Avenue
Chcago, Illinois 60616

Professor of Surgery
Pritzker School of Medicine
University of Chicago
Chicago, Illinois 60637

21 Sep 1978

63 p.

78 10 16 042

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Treatment of Hemorrhagic Shock Saline Vs. Colloid Solution		5. TYPE OF REPORT & PERIOD COVERED Annual May 1, 1978-April 30, 1979
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Gerald S. Moss, M. D.		8. CONTRACT OR GRANT NUMBER(s) N00014-75-C-1086
9. PERFORMING ORGANIZATION NAME AND ADDRESS Michael Reese Hospital 2929 South Ellis Avenue Chicago, Illinois 60616		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR-105-539
11. CONTROLLING OFFICE NAME AND ADDRESS Michael Reese Hospital 2929 South Ellis Avenue Chicago, Illinois 60616		12. REPORT DATE September 21, 1978
		13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution of this report is unlimited.

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Pulmonary Interstitial Edema
Albumin
Radioautography
Hemorrhagic Shock
Surfactant
Sympathectomy

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Electron microscopic studies show pulmonary interstitial edema in the hemorrhaged baboon results from the use of 5% serum albumin as a resuscitative fluid. Radioautography of radioiodinated albumin in the pulmonary interstitium is performed. Fluorescent micrography of Evans Blue Albumin is planned.

DD FORM 1473 1 JAN 73 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

TABLE OF CONTENTS

A. Studies Completed This Year

   1. Introduction

INTRODUCTION

The thrust of our research has been to explore clinical surgical problems that would have great relevance to military medicine. For example, we have just completed a series of studies involving hemorrhagic shock in the baboon followed by clinical studies in man. These studies indicated that Ringer's solution is equally effective as albumin solution for initial resuscitation from hemorrhage, yet albumin costs approximately 100 times as much as Ringer's solution. The decision to use Ringer's rather than albumin would have great impact on military medicine.

In our current project, we are developing new ways to examine what happens to lungs in septic shock. We believe septic shock remains an unsolved clinical problem. Our long range goal is to examine changes in interstitial albumin and sodium plus changes in alveolar surfactant during septic shock. We also hope to compare the pulmonary effects of Ringer's lactate versus albumin infusion in septic shock in baboons and then in man.

A. Studies Completed This Year

    2.   Changes in Lung Ultrastructure Following Heterologous
and Homologous Serum Albumin Infusion in the Treatment
of Hemorrhagic Shock.

CHANGES IN LUNG ULTRASTRUCTURE FOLLOWING HETEROLOGOUS AND HOMOLOGOUS SERUM ALBUMIN INFUSION IN THE TREATMENT OF HEMORRHAGIC SHOCK

Gerald S. Moss, M. D.

Tapas K. Das Gupta, M. D.

Richard Brinkman, M. D.

Lakshman Sehgal, Ph.D.

Bobbie Newsom, B. S.

We have previously shown by electron microscopic studies that interstitial pulmonary edema can be observed in the lungs of baboons infused with human serum albumin in the treatment of hemorrhagic shock.[7] Similar treatment with Ringer's lactate solution produces no such changes in this animal preparation. The development of interstitial pulmonary edema following human serum albumin administration in the baboon has several possible explanations. One is that the infused albumin accumulates in the lung interstitium. Another is that the changes seen in the lung ultrastructure are related to the infusion of a heterologous protein solution.

The object of this study was to compare the ultrastructural pulmonary effects of homologous and heterologous serum albumin infusion in the treatment of hemorrhagic shock in baboons.

Methods and Materials

Thirteen baboons were the test animals. Two were controls, two were sacrificed after hemorrhage, three were sacrified after each of the following therapies - Ringer's lactate infusion, human serum albumin infusion, and baboon serum albumin infusion.

Each animal was tranquilized while still in his cage with phencyclidine hydrochloride (0.8 mg/kg). Under local anesthesia, a siliconized plastic catheter was placed in one femoral artery for blood withdrawal and arterial pressure measurements. Another catheter was inserted into the inferior vena cava via the femoral vein for infusion therapy and venous pressure measurements. An endotracheal tube was inserted and the animal was then loosely restrained in the prone position.

Hemorrhage: The arterial pressure was reduced to 60 mm Hg for one hour and then to 40 mm Hg for a second hour by femoral artery bleeding into a plastic bag containing 67.5 cc of citrate-phosphate-dextrose.

Resuscitation: Each animal was resuscitated over a four-hour period. A particilar test fluid was given in volumes sufficient to restore and maintain arterial pressure and venous pressure to prehemorrhage levels.

Electron Microscope: At the end of each study, the pulmonary artery was catheterized via a medium sternotomy under sodium pentobarbital anesthesia. The lungs were fixed by in vivo dual perfusion via a pulmonary artery and bronchus by a technique previously described.[5] The perfusate was potassium pryoantimonate. This solution forms an electron-opaque complex with sodium, thus providing a method for identifying the distribution of pulmonary interstitial sodium. This is an established technique which has been utilized in prior studies.[3]

Fixed tissues were dehydrated in acetome and embedded in Araldite or Epon-812. Ultra-thin sections were mounted on a copper grid, stained with uranyl acetate, and viewed under an RCA EMU 312-4 microscope. Each specimen was read and evaluated by one of us (TKD) without knowledge of the treatment regimen.

Purification of Baboon Serum Albumin: The procedure, a modification of the
Cohn fractionation technique[2] was adapted to permit the purification of
400 ml of citrated plasma at a time.   Purity of the preparation was checked
by electrophoresis, which showed a single uniform peak (Figure 1).

Results

A. Clinical Observations

All 13 animals survived shock and resuscitation. Table 1 shows the
volumes of fluid infused in the gruops. Animals in the Ringer's lactate
group received a significantly larger volume of fluid than those that
received albumin ($p < 0.05$).

B. Electron Microscopic Findings

Normal Lung: In the normal baboon lung (Figure 2a), the thin portion of
the alveolar capillary membrane is composed of an epithelial and endothelial
layer with an intervening narrow interstitial zone. In the thicker portion of
the alveolar caipliary membaran (Figure 2b), the interstitial zone becomes
more complex. This zone contains collagen fibers which are of special interest,
since the earliest electron microscopic findings of interstitial edema is
dispersion of the individual fibers. In the intersitium, occasional sodium
precipitates can be seen in relation to individual collagen fibers,
indicating an inherent affinity between collagen and sodium.

Effects of Hemorrhagic Shock: Striking alterations in lung tissue were seen following hemorrhagic shock, the most profound being in the interstitium (Figure 3a). Here, large accumulations of edema fluid were noted. The intersititial edema fluid stained intensely, indicating the presence of sodium (Figure 3b). In addition, the concentration of sodium in and around the dispersed collagen fibers was remarkably increased.

C. Effects of Resuscitation:

Ringer's Lactate: The lung tissue of animals resuscitated with Ringer's lactate was practically indistinguishable from that of normal lungs (Figure 4a). The evidence for interstitial edema was minimal. There were few empty spaces, the smudge areas were rare, and the collagen fibers were tightly packed as in the normal state. Sodium precipitates (Figure 4b) were occasionally seen in relationship to the collagen fibers.

Human Serum Albumin: In the lungs from animals resuscitated with human serum albumin (Figure 5a), the tissues appeared similar to those seen in the untreated animals subjected to hemorrhage. Interstitial edema persisted, as manifested by empty spaces in the interstitium, smudging, and collagen fiber dispersion. In addition, abnormal concentrations of interstitial sodium (Figure 5b) could be seen especially in proximity to collagen fibers.

Baboon Serum Albumin: We could find no difference in the electron microscopic appearance of lungs from animals treated with baboon serum albumin and those treated with human serum albumin. Evidence of interstitial

edema was present in all sections studied (Figure 6a). There were multiple empty spaces observed in the interstitium. Smudging was frequently observed. There was gross dispersion of interstitial collagen fibers. Increased concentrations of sodium (Figure 6b) were noted in the edema fluid, especially in proximity to collagen fibers.

Discussion

In this study, baboons resuscitated from hemorrhagic shock with either homologous or heterologous serum albumin developed ultrastructural evidence of pulmonary edema. Animals resuscitated with Ringer's lactate solution showed no major changes in lung ultrastructure despite the significantly larger infusion volumes required by this group. These results suggest that the infused albumin accumulated in the baboon lung interstitium and produced interstitial edema.

These results are difficult to reconcile with the traditional Starling hypothesis.[8] The hypothesis states that the movement of water across a semipermeable membrane is dependent upon the algebraic sum of the hydrostatic and colloid osmotic pressures on both sides of the membrane. If this hypothesis were correct for the lung, the infusion of albumin during hemorrhagic shock would maintain plasma colloid osmotic pressure and therefore inhibit the movement of plasma water into the lung interstitium. On the other hand, the infusion of Ringer's lactate will lower plasma oncotic pressure and therefore favor the filtration of plasma water into the lung interstitium.

The best explanation for the descrepancy between our results and the
predicted results based on the Starling hypothesis involves the permeability
of the pulmonary capillary membrane to albumin. The Starling hypothesis
assumes that the pulmonary capillary membrane is impermeable to albumin. It
is now clear that this is incorrect. In fact, large amounts of albumin
pass through the pulmonary capillary membrane and are cleared from the lung
presumably through the lymphatic system. The evidence for this point of
view is as follows:

In studies in sheep[1] and baboons[10], lung lumph was collected and
analyzed for albumin levels. If the pulmonary capillary membranes were
impermeable to albumin, lung lymph albumin levels should be low compared
to plasma. In both sheep and baboons, the lung lymph albumin levels were
approximately 80% of the plasma value, indicating a high degree of
permeability for albumin.

The effects of elevated pulmonary microvascular pressure on pulmonary
lung albumin in sheep was reported by Brigham.[1] When lung vascular pressures
were increased, lung lymph flow increased, and lung lymph albumin levels fell.
The fall in lung interstitial albumin levels offset the effect of increased
intravascular pressure. Thus, the high level of basal pulmonary interstitial
ablumin represents a safety factor against increased intravascular pressure.

The effect of reduced plasma colloid osmotic pressure on pulmonary
lymphatic kinetics was also studied. Lung lymph was collected from baboons
subjected to profound plasmaphoresis with Ringer's lactate.[10] Lung lymph
albumin levels declined in a similar fashion to plasma levels. The lowest

levels were approximately 1 gram % for both fluids. In addition, lung lymph flow increased fourfold. Despite a 15% gain in body weight during the plasmaphoresis, there was no evidence of pulmonary edema. There were no changes from control levels in blood gases, intrapulmonary shunt, dynamic compliance, or lung water content. The results of this study illustrate again the safety factor provided by high levels of pulmonary interstitial albumin in the normal state. A fall in plasma colloid osmotic pressure is offset by a corresponding decline in pulmonary interstitial colloid osmotic pressure.

The effect of lowered plasma colloid osmotic pressure on lung function in man has been reported.[9] A group of 43 patients undergoing major abdominal aortic surgery was resuscitated with either Ringer's lactate or 5% albumin solution. The relationship between changes in the gradient between colloid osmotic pressure (COP) and pulmonary capillary wedge pressure (PCWP) versus intrapulmonary shunt was studied. The base line gradient (COP-PCWP) was nine torr and the intrapulmonary shunt was 15%. No relationship could be found after resuscitation between changes in gradient and changes in intrapulmonary shunt. In several patients resuscitated with Ringer's lactate, the gradient became negative as the colloid osmotic pressure declined and PCWP remained constant. Despite marked weight gain and peripheral edema in these patients, no evidence of pulmonary edema was noted. Presumably, increased lymphatic clearance of pulmonary interstitial albumin protected the lungs from the effects of reduced plasma oncotic pressure.

In another clinical study, Lowe et al., studied 137 patients undergoing laparotomy for trauma who were resuscitated with either Ringer's lactate or 5% albumin solution. Since red cell losses were replaced with washed red cells, the patients in the Ringer's lactate group were resuscitated without any albumin. No differences were noted between the two treatment groups in death rates or in the incidence of respiratory failure.

Changes in lung capillary permeability to albumin have also been studied. Lung lymph was collected from sheep treated with either histamine or pseudomonas bacteria.[1] The lung lymph flow increased in a fashion similar to the results of the increased vascular pressure studies. However, the concentration of albumin in the lung lymph remained unchanged, in contrast to the results of the increased intravascular pressure studied. These results suggest that the safety factor of declining pulmonary interstitial colloid osmotic pressure is lost when the pulmonary capillary permeability to albumin is increased.

Increased capillary permeability was demonstrated clinically by Robin et al.[6] They studied alveolar fluid contents in patients suffering from pulmonary edema and reported the levels of albumin and fibrinogen in the alveolar fluid were in equilibrium with plasma levels. High molecular weight Dextran (5000,000 M.W.), when injected intravenously, appeared in the alveolar fluid within minutes. The authors termed this "capillary leak symdrome".

In summary, we found that homolouous serum albumin, when infused into the hemorrhaged baboon, produced ultrastructural evidence of interstitial edema. The best explanation for these findings is that the infused albumin accumulates in the lung interstitium.

14

Summary

The object of this study was to compare the ultrastructure pulmonary
effects of the infusion of homologous and heterologous serum albumin
solution in the treatment of hemorrhagic shock in baboons.

Adult baboons subjected to hemorrhagic shock were resuscitated
with either baboon serum albumin, human serum albumin, or Ringer's
lactate solution. The lungs were fixed in vivo with potassium pyroantimony, a
solution which produces electron dense interstitital precipitation of
sodium.

The lungs from animals resuscitated with baboon serum albumin
showed evidence of interstitial edema, including dispersion of collagen
fibers, interstitial smudging and increased interstitial sodium con-
centrations. Similar changes were soon following human serum albumin
infusions. Lung tissue from animals treated with Ringer's lactate solution
showed minimal changes from normal.

These results suggest that interstitial pulmonary edema develops
after either homologous or heterologous serum albumin infusion in the
treatment of hemorrhagic shock in baboons.

FIGURE 1:
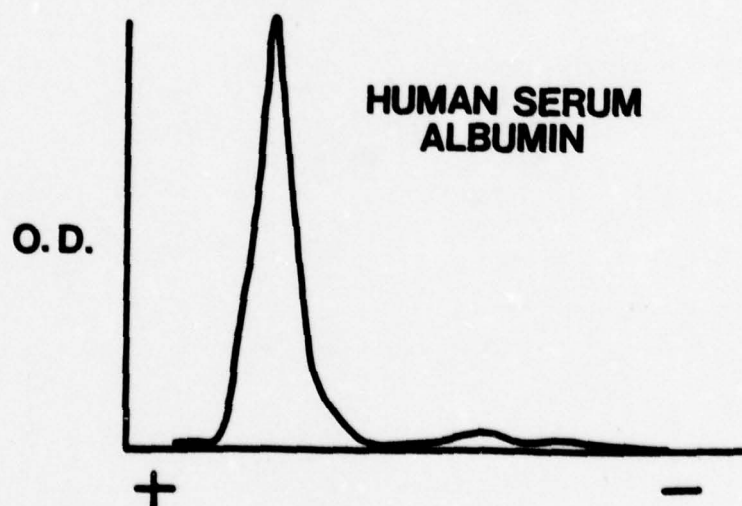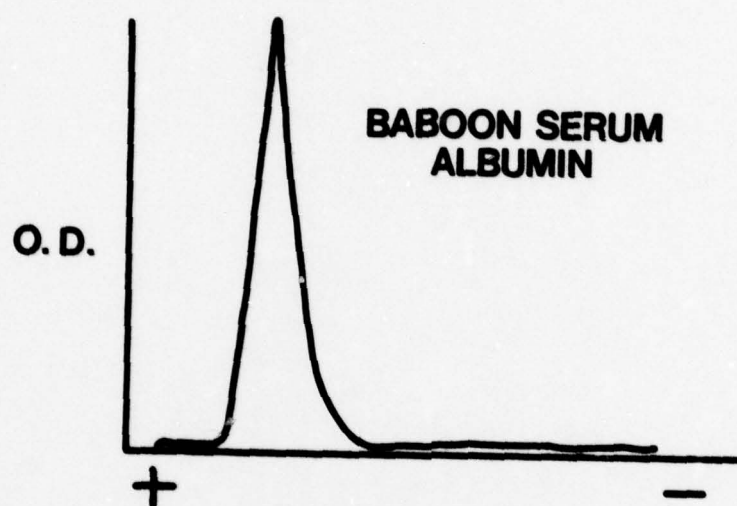
FIGURES 2a and 2b:

MOSS ET AL.
"Changes in Lung Ultrastructure..."

17

FIGURES 3a and 3b:

MOSS ET AL.
"Changes in Lung Ultrastructure..."

18

FIGURES 4a and 4b:

MOSS ET AL.
"Changes in Lung Ultrastructure..."

FIGURES 5a and 5b: MOSS ET AL.
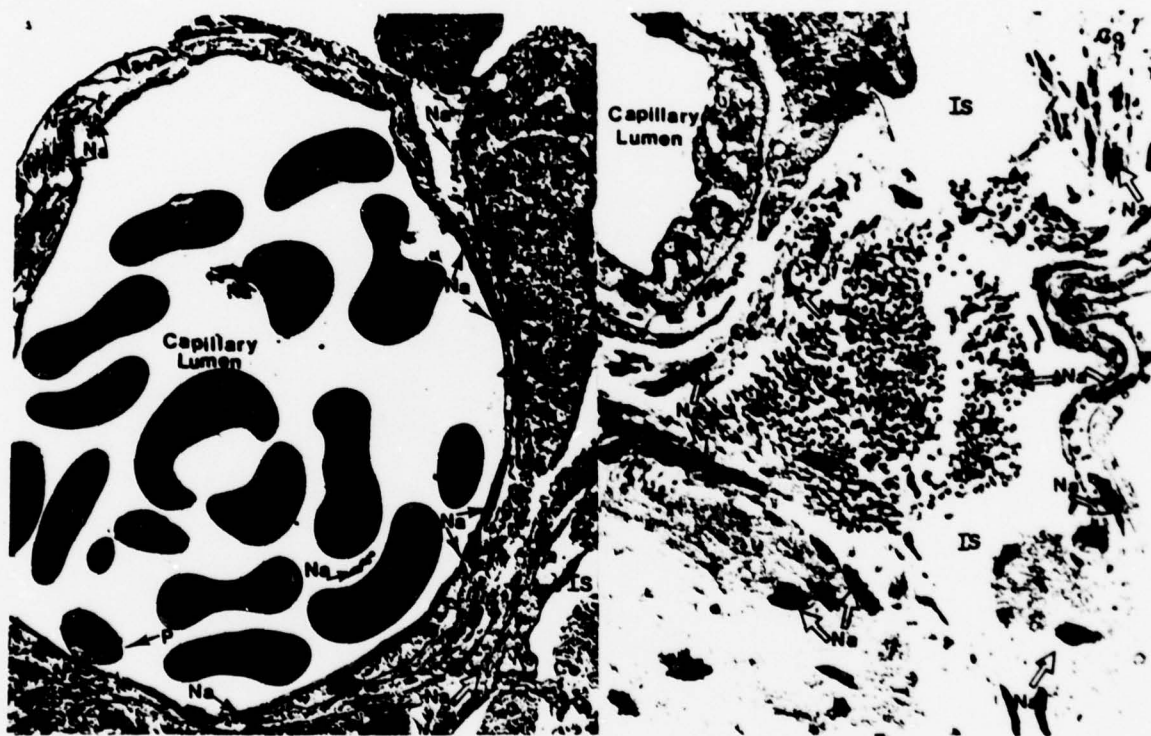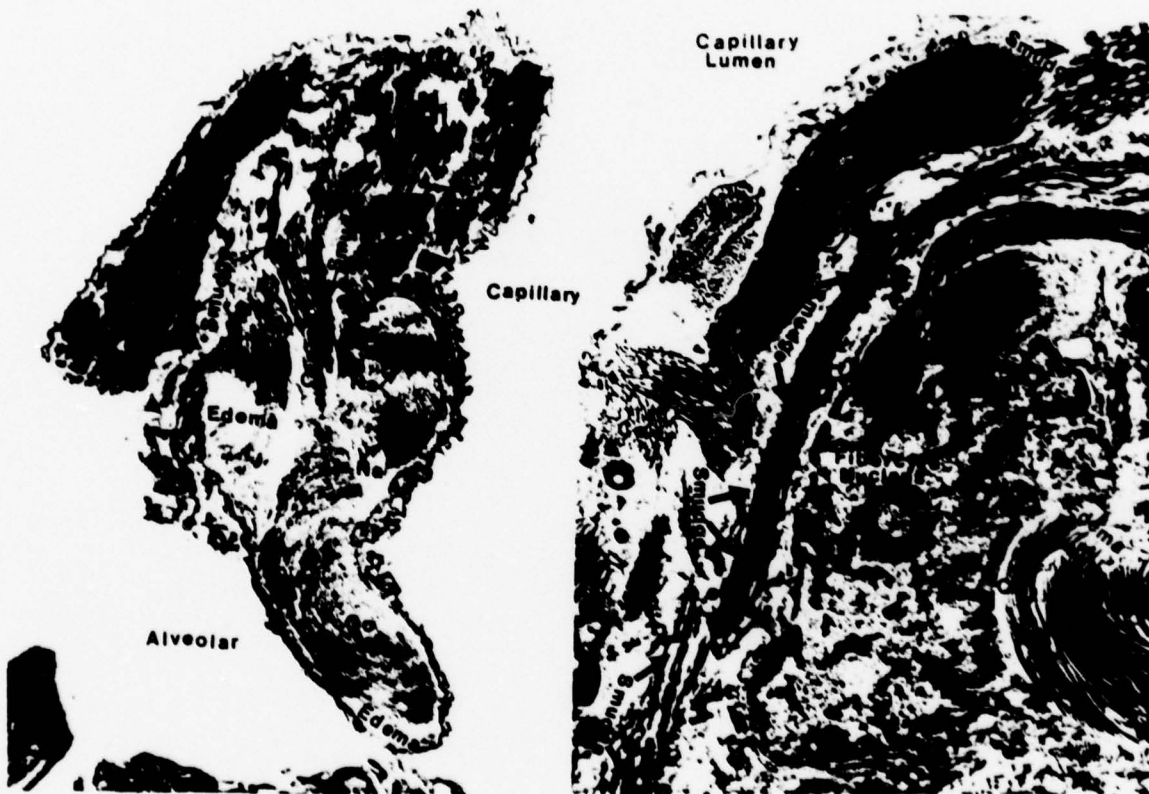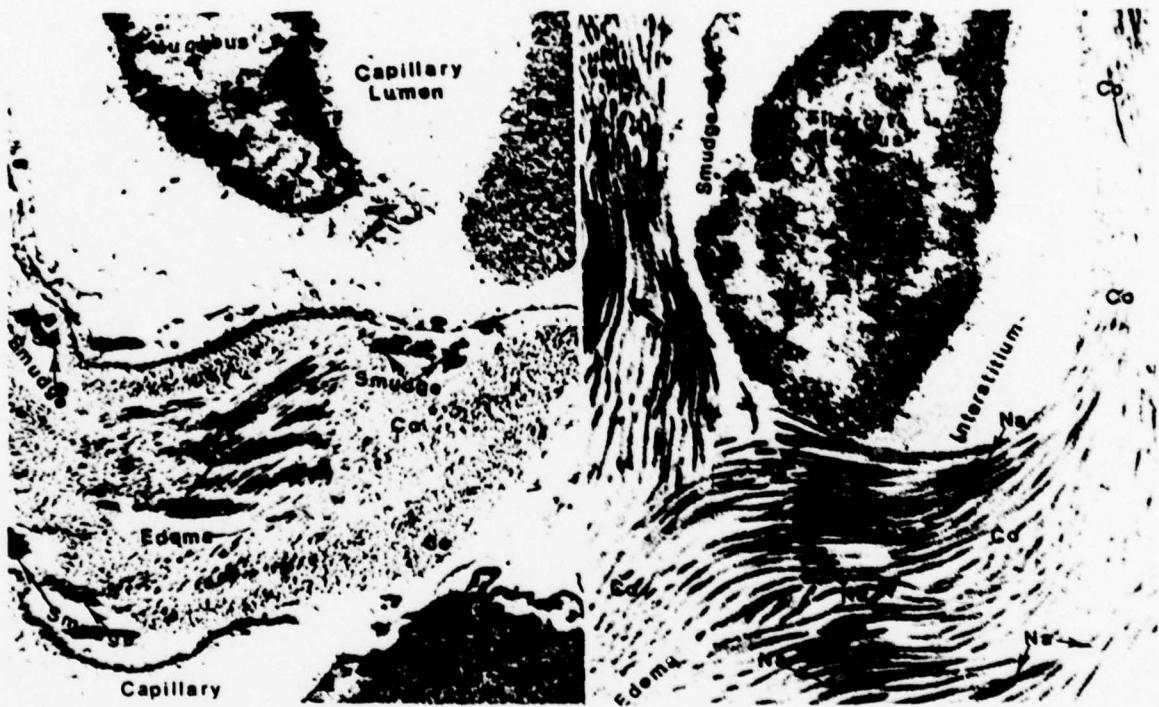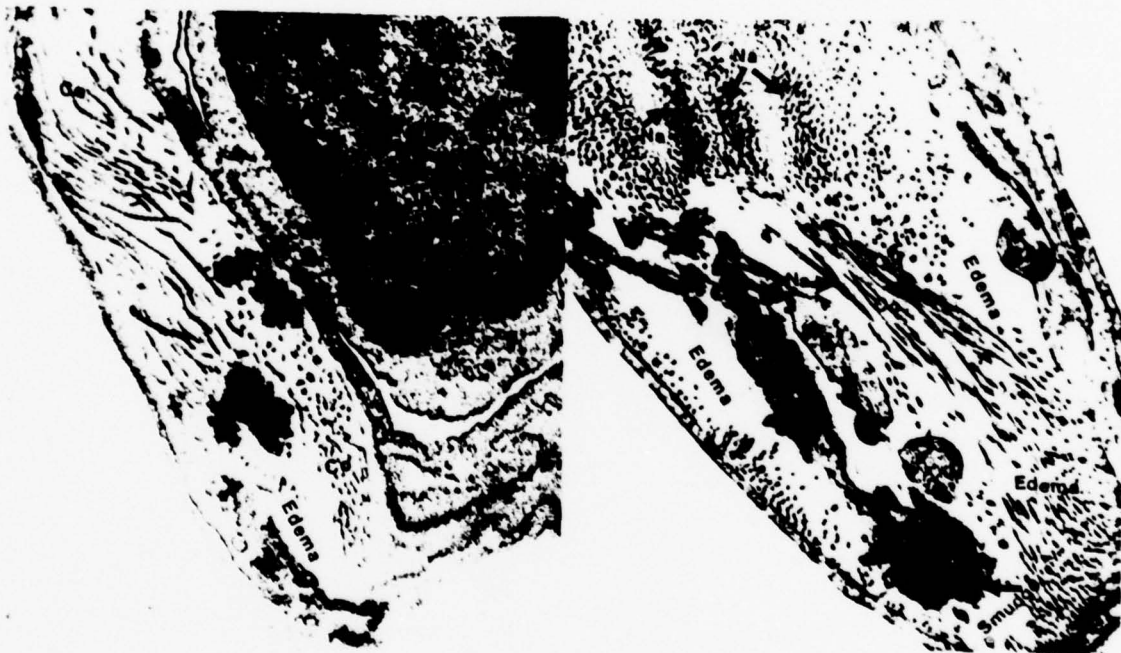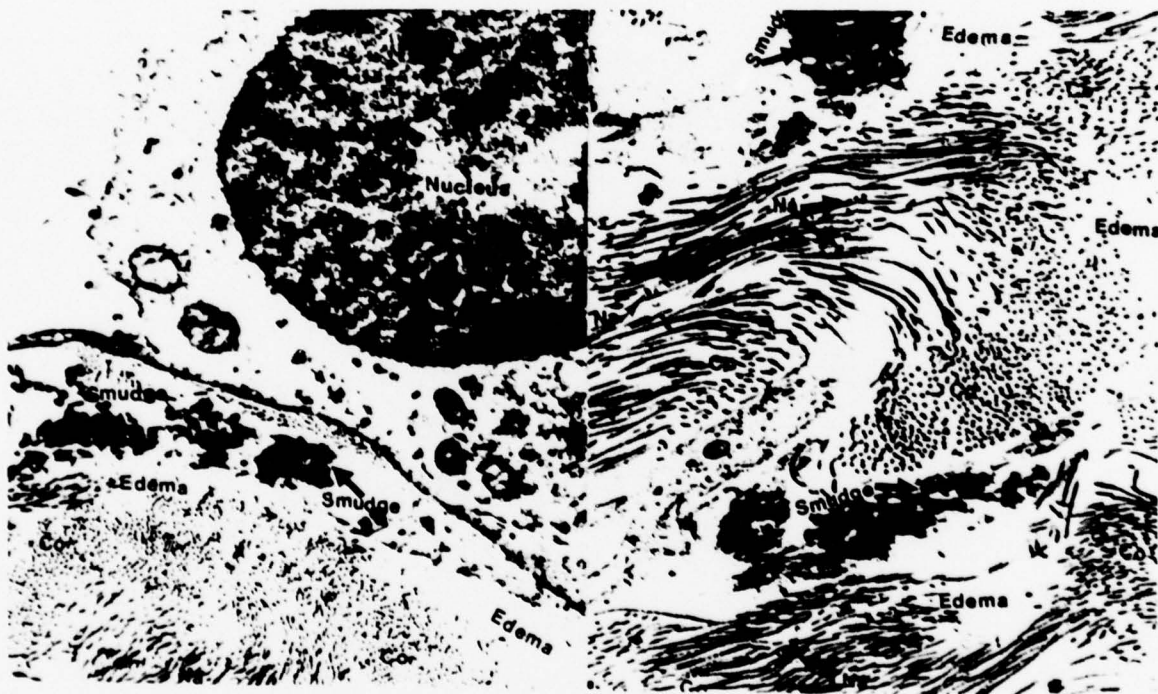   "Changes in Lung Ultrastructure..."

FIGURES 6a and 6b: MOSS ET AL.
                                          "Changes in Lung Ultrastructure..."

A. Studies Completed This Year

   3. Alterations in Pulmonary Surfactant Following Sympathectomy.

Surfactant can be defined as the surface-active material which forms the alveolar layer. The dynamic properties of surfactant which contribute to the normal mechanics of the lung are known as the surfactant system.[1] Without surfactant, the alveolus would behave like an ordinary bubble and would collapse with the high surface tensions generated by the contracting lung.

Today it is well accepted that an impairment of the surfactant system is involved in several types of pulmonary pathology; for example, the infant respiratory distress syndrome (hyaline membrane disease)[2] and the adult respiratory distress syndrome[3], associated with cardiopulmonary bypass, lung transplantation, trauma, pancreatitis, or long-term mechanical ventilation.

Of these, only the infant respiratory distress syndrome has been clearly connected with a surfactant deficiency. In the others, a disturbance in the surfactant system of the lung is recognized, but the exact mechanism by which this disturbance is mediated still remains unknown. Therefore, a systematic investigation of the morphologic, physiologic, and biochemical properties, along with the controlling factors in surfactant function, is in order.

The study of surfactant began in 1929 when von Neergaard[1] recognized the need for a surfact-active substance in the alveolus. He compared fluid-filled lungs with air-filled lungs and found that they contracted differently. This difference he attributed to what he believed were surface forces in the air-filled lung. A surface force is created when two substances of different densities, such as air and alveolus, come into contact with each other. However, von Neergaard was unable to make exact measurements of these surface forces or describe in detail the surface-active substance which he thought was present in the lung.

Little experimentation on the surfactant system was done for the next 25 years, until Pattle[4] in 1955 described a surface-active material in the lung. He washed animal lungs with saline and found that he obtained a foam which possessed unique characteristics. The bubbles of the foam were highly resistant to antifoaming agents and were of a constant size. When the bubbles were placed in air-free water, they disappeared, thereby demonstrating their permeability to air, but they also left behind in the water a transparent film or "ghost" which Pattle interpreted as being the surface-active material which had been lining the bubble of pulmonary foam.

Following the work of Pattle, Clements[5] in 1957 made the next significant contribution in the field of surfactant research. He was actually able to measure the surface tension of the lung tissue. Clements demonstrated that the surface-active material found in the lung was capable of reducing the surface tension of the air-lung interface when the alveolus was compressed or contracted.

The research of von Neergaard, Pattle and Clements established the basic principles of surfactant research. They found that there was a powerful surface-active material in the lining of the alveolus which was capable of influencing the surface tension in the alveolus. By altering the surface tension, the surfactant system was able to affect the mechanical properties of the lung.

Having established these principles, research was then directed to the morphology and biochemistry of the surfactant system. Using the electron microscope, lung tissue was studied extensively. At the level of the alveolus two types of epithelial cells were identified. The type I pneumocyte (Type A) is a small cell (0.7 microns in diameter) located directly on the alveolus (Figure 1). The function of this cell remains unknown. The type II (type B)

or granular II pneumocyte, is larger (7-14 microns) and its cytoplasm is filled with large cytosomes or lamellated bodies.[6] Studies now indicate that this granular II pneymocyte may be an integral part of the surfactant system. Examination of various mammalian fetuses (for example, the rabbit fetus) shows that there is simultaneous development of both the alveolar lining layer and surface activity in the lung several days after the appearance of lamellated bodies within the granular II pneumocyte.[7] Research in ontogeny also indicates that a surface active pulmonary lining is present in those animals with granular pneumocytes.[1]

In addition to defining cellular structure in the lung, the electron microscope was used to demonstrate an alveolar acellular lining layer. This layer is located between the alveolar epithelial cells and the air in the alveolus. The lining layer was described by Gil and Weibel [8,9] and it stains with osmium tetrozide, appearing as a dark alveolar lining under the electron microscope. Bolande and Klaus[10] were able to demonstrate a similar alveolar lining with their fluorescent studies on the light microscopic level.

Extensive biochemical analyses of the alveolar lining layer demonstrated that the major surface active material was phospholipid, dipalmitoyl lecithin.[1] Buckingham and co-workers,[11] using various radicactive isotopes, then began to label fatty acids and determined the symthesis of phospholipid in the lung was relatively rapid. After synthesis in the lung, the phospholipid could then be traced to a location in the alveolar lining layer. It was found that radio-actively labeled precursor of lecithin appeared first within the granular II pneumocyte and then in the alveolar lining layer.

Although thorough morphologic, physiologic, and biochemical experiments have been performed, the control mechanism of the surfactant system has not

yet been discovered. There has been much speculation during the past
ten years as to a neural or a neurohumoral control of surfactant production.
This work has mainly been directed at the granular II pneumocyte since radio-
isotope studies indicate this cell as the probable source of pulmonary
phospholipid. However, no nerve endings have yet been described in direct
contact with these cells. There has been some research directed to the theory
that the granular pneumocyte resembles a secretory cell and may be under the
control of the vagus nerve.[12] However, the experiments on the vagus have been
inconclusive and the effect of the parasympathetic nerves on the granular II
pneumocyte and the surfactant system remains uncertain. In contrast, few
studies have been done on the relationship between the sympathetic nervous
system and surfactant. Some investigations have attempted to link the
sympathetic system to pulmonary insufficiency in conditions such as head
trauma.[13, 14] Wyszogrodski et al.[15] suggested the use of catecholamines in the
treatment of respiratory insufficiency in premature rabbits. However, none
of this research has defined the exact role of the sympathetic system in
surfactant production.

The purpose of this study was to determine the relationship between
the nervous system and both the granular II pneumocyte and the phospholipid
lining of the alveolus. In order to treat the clinical conditions resulting
from surfactant dysfunction, it is first necessary to understand what controls
the surfactant system of the lung.

MATERIALS AND METHODS

Fifty neonatal Sprague-Dawley rats of either sex were divided equally
into two groups. Beginning within six hours after birth, the experimental

group received intraperitoneal injections daily for eight days of 50 g/gm of body weight of 6-hydroxydopamine, an agent that selectively destroys adrenergic nerve endings and produces chemical sympathectomy.[16] The control intervals by cervical dislocation between the ages of three and eight weeks. Lung tissue was obtained immediately for light and electron microscopy. The tissue for electron microscopy was fixed in 4% glutaraldehyde, stained with osmium tetroxide, and processed.

Additional lung tissue was obtained for phospholipid staining by the method of Weller, Bayiss and Abdulla [17], developed in 1965. This technique utilized ammoniacal silver nitrate and hydroxamic acid to localize phospholipids as a final reaction product of dark silver granules.

The glutaraldehyde-fixed tissue was cut between 30-5- microns and treated in Gallya's ammoniacal silver nitrate solution for 24 hours. The sections were then stained in an alcoholic phosphotungstic acid solution and embedded in Araldite. Thin sections were then cut and mounted for the electron microscope. Tissue for light microscopy was routinely mixed in neutral formaldehyde, processed and cut in 7-micron sections. Both hematoxylin-eosin and periodic acid-Schiff stains were used.

Lung tissue was also analyzed for total lipids, phospholipid content and fatty acids. Lipids were extracted by Folch's method (2:1 Chloroform-methanol). Water-soluble nonlipid contents were removed and the amount of lipid was determined gravimetrically. Phospholipid assay was done by determining the amount of phosphorus in a specific volume (Bartlett's method). Fatty acids were determined by gas chromatography.

In evaluating the data from the experiment concerning the lamellated bodies of the sympathectomized animals, a scoring system was developed.

The scores ranged from one to five based on the content and organization of the lamellated bodies. Seven animals were selected from the control group and five from the sympathectomized group. Fifty cells with 400 lamellated bodies were scored from each group. The p value was 0.005.

RESULTS

Control Group:

The low power views from the control lungs showed well-preserved alveoli, normal capillaries containing red blood cells, and intact pulmonary interstitium. The epithelial cells occupied their usual position on the alveolar lumen and their membranes were smooth. The granular II pneumocyte had its characteristic oval eccentric nucleus and its granular cytoplasm, with well-developed endoplasmic reticulum. The most prominent cytoplasmic organelle was the lamellated body which contained concentric layers of densely osmiophilic material. Also, numerous mitochondira and multivesicular bodies could be seen. The multivescular bodies were small clusters of tubules which were distributed throughout the cytoplasm.

Structures in the cytoplasm of the granular II pneumocytes resembling transition forms between the numerous multivesicular bodies and the lamellated bodies were encountered. The lamellated bodies were located in various positions from the perimuclear area to the periphery of the cytoplasm. Some lamellated bodies were found to be covered with only a thin rim of cytoplasm. Others appeared to be extruded into the alveolar space.

The lamellated bodies were also found free in the alveolus directly adjacent to the granular II pneumocytes. Occasionally a lattice arrangement was noted in the alveoli. This ordered structure is known as tubular myelin

figure and occurs in normal animals.[9]

Control Group Phospholipid Stain. An electron micrograph from a control
animal demonstrated the normal distribution of phospholipid.[18] With this
staining technique the phospholipid occurred as small dark granules. Within
the lamellated bodies, the phospholipid was distributed as fine granules around
the periphery of the body. Lining the alveolus, the phospholipid seemed to
occur as a rather continuous line of small granules.

Control Group Biochemical Analysis. The phospholipid phosphorus per
gram of total lipid in the control lungs showed a mean of 656 micromoles with
SD 62.

Sympathectomized Group:

Peripheral and hilar axonal degeneration validated the technique of
sympathectomy in the 6-hydroxydopamine treated lungs. The alveoli were well
preserved, but contained bizarre, elongated tubular myelin forms the
capillaries were intact. However, many of the granular pneumocytes showed
extensive cytoplasmic degratation with a dilation of the endoplasmic
reticula. The most prominent structural alteration following sympathectomy
occurred in the lamellated bodies. In a high power view of a granular
pneumocyte the lamellated bodies demonstrated a thinning of the lamellae,
a loss of the concentric pattern within the body, and a decrease in the
osmiophilic content. Some of the lamellated bodies appeared almost empty.

In an attempt to quantitate the differences observed in the lamellated
bodies, the following scoring system was developed. A lamellated body with
a score of five had thick concentric rings of dense osmiophilic material.
A score of four represented a thinning of the lamellae. A score of three
meant a loss of the concentric pattern and with a score of two, only peripheral

coarse osmiophilia remained. A score of one was a lamellated body which was almost empty, with only a thin rim of osmiophilia.

Using this scoring system, the following data was accumulated (Table). The mean score form the control animals was 4.43 and the mean in the sympathectomized group was 3.77 was a p value of < 0.005. These figures indicate that there was a significant difference in the lamellated bodies of the granular II pneumocyte following sympathectomy.

Sympathectomized Group Phospholipid Stain. After sympathectomy, three major patterns of phospholipid distribution were noted. In one group, the lamellated bodies of the granular II pneumocyte contained large clumps of phospholipid with some of the reaction product located in the center of the body, with a loss of even distribution of dark granules along the alveolus. In the second group the lamellated bodies within the granular pneumocytes were empty, and there was absence of the dark phospholipid granules lining the alveolus. In the third category, the phospholipid lining was clumped and fragmented in appearance.

Sympathectomized Group Lipid Biochemical Analysis. The phospholipid phosphorus per gram of total lipid in the sympathectomized lungs showed a mean of 892 micromoles with SD 31 (P≤ 0.001). Analysis of fatty acids demonstrated that unsaturated fatty acids were unaffected by sympathectomy.

DISCUSSION

The control animal lungs in this study were found to have the typical morphology of the rat lung. The only variations concerned the lamellated bodies of the granular II pneumocyte. The lamellated bodies seemed to originate from the multivesicular bodies and numerous transition forms were

seen in the control animals. Once completely formed, the lamellated
bodies appeared to migrate to the periphery of the cell. From there,
they were extruded into the alveolus. In addition to lamellated bodies,
other structures such as the lattice tubular myelin figures were found in the
alveoli and could represent another form of the lamellated phospholipid
material in the alveolus. From these observations, it can be postulated that
the lamellated body arises from the multivesicular body, migrates through
the granular pneumocyte and is extruded into the alveolus. Once in the
alveolus, the phospholipid of the lamellated body becomes applied to the
alveolus as surfactant---the alveolar lining layer.

The hydroxylamine staining technique showed the control lungs to contain
a fine, even distribution of phospholipid both in the lamellated bodies and
along the surface of the alveolus.

Following sympathectomy there were no transitional forms between the
multivesicular and lamellated bodies. Only an occasional lamellated body
was found to be extruded into the alveolus and there were no structured
tubular myelin figures. Instead, there were bizarre, elongated tubular
myelin forms in the alveoli. There were also changes within the lamellated
bodies consisting of a decrease in both the osmiophilic content and organization
of the bodies.

Using the scoring system previously described, it was possible to
quantitate these changes and establish that there is a significant
difference in the lamellated bodies after sympathectomy. In addition, there
was a change of the phospholipid pattern within the lamellated bodies
and fragmentation of the alveolar lining layer.

From these observations, it can be seen that sympathectomy in the rat produces changes in the granular II pneumocyte and the surfactant layer of the lung. The mechanism for these changes is still undetermined but there are several unexplored possibilities. There may be direct sympathetic innervation of the granular pneumocyte but as yet there have been no nerve endings described in contact with this particular cell. There could also be an indirect effect on either the pulmonary vasculature or bronchi. Since both these structures have sympathetic innervation, it might be possible to influence granular pneumocyte function through its blood supply or ventilation. The third mechanism is that of a sympathetically innervated neurosecretory cell on which the granular II pneumocyte is hormonally dependent. A type of neurosecretory cell has already been described in the lungs of several mannals, including man.[19, 20] These cells are known as neuroepithelial bodies and their function in the lung has not yet been determined.

The most likely of these three mechanisms is that of a neurosecretory type since the change noted in the granular pneumocyte is a selective one. The sympathectomy affects the pattern of phospholipid distribution in the lamellated bodies and along the alveolus. This suggests a disturbance in fatty acid metabolism and transport within the granular pneumocyte. Such a defect in the surfactant system would be difficult to create by simple denervation. These observations imply that normal surfactant production may be dependent on a neural or neurohumoral mechanism.

This study has both research and clinical implications. If it is possible to produce an alteration in the surfactant system by performing a sympathectomy it may be practical to use a denervated lung as a model

to study surfactant production. In many clinical syndromes such as the infant and adult respiratory distress syndromes, a defect in the surfactant system has been hypothesized. However, until the mechanism of surfactant synthesis and maintenance is understood, it will continue to be impossible to treat these forms of respiratory insufficiency. By defining exactly where the defect occurs in a deranged surfactant system such as the one created in this study, it may be possible to correct this defect by administering a sympathetic agent such as a biogenic amine.

REFERENCES

1. Scarpelli, E.M.: The Surfactant System of the Lung. New York,
    Lea and Febinger, 1968.

2. Farrell, P.M. and Avery, M.E.: Hyaline Membrane Disease. Am. Rev.
    Resp. Dis., Vol. III:657-688, 1975.

3. Heinemann, H.O.: Surfactant of the Lung. Adv. Int. Med. 14:83-105, 1968.

4. Pattle, R.E.: Properties, Function and Origin of the Alveolar Lining
    Layer. Nature 175:1125-1126, 1955.

5. Clements, J.A.: Surface Tension of Lung Extracts. Proc. Soc, Exp.
    Biol. Med. 95:170-172, 1957.

6. Sorokin, S.P.: A Morphological and Cytochemical Study on the Giant
    Alveolar Cell. J. Histochem. Cytochem. 14:884-897, 1968.

7. Kikkawa, Y, Motoyama, E.K., Gluck, L.: Study of the Lungs of Fetal
    and Newborn Rabbits: Morphological, Biochemical, and Surface Physical
    Development. Amer. J. Path. 52:177-210, 1968.

8. Weibel, E.R. and Gil, J.: Electron Microscopic Demonstration of an
    Extracellular Duplex Lining Layer of Alveoli. Resp. Physiol. 4:42-57, 1968.

9. Gil, J. and Weibel, E.R.: Improvements in Demonstration of Lining
    Layer of Lung Alveoli by Electron Microscopy. Resp. Physiol. 8:13-36,
    1969, 1970.

REFERENCES (Continued)

10. Bolande, R.P., and Klaus, M.H.: The Morphological Demonstration of an
    Alveolar Lining Layer and Its Relationship to Pulmonary Surfactant.
    Am. J. Path. 45:449-463, 1964.

11. Buckingham, S., Heinemann, H.O., Sommers, S.C., and McNary, W.F.:
    Phospholipid Synthesis in the Large Pulmonary Alveolar Cell. Amer.
    J. Path. 48:1027-1041, 1966.

12. Goldenberg, V.E., Buckingham, S., and Sommers, S.C.: Pilocarpine
    Stimulation of Granular Pneumocyte Secretion. Lab. Invest. 20:147-158,
    1969.

13. Beckman, D.L., Bean, J.W., and Baslock, D.R.: Sympathetic Influence
    On Lung Compliance and Surface Forces in Head Injury. J. Appl. Physiol.
    30:394-399, 1971.

14. Beckman, D.L., et al.: Sympathetic Factors in Acute Pulmonary Injury.
    Chest 65:385-405, 1974.

15. Wyszogrodski, I., Taeusch, H.W., and Avery, M.E.: Isoxsuprine-induced
    Alterations of Pulmonary Pressure-Volume Relationships in Premature
    Rabbits. Am. J. Obstet. Gynecol. 119:1107-1111, 1974.

16. Thoenen, H. and Tranzer. J.P.: Chemical Sympathectomy by Selective
    Destruction of Adrenergic Nerve Endings with 6-Hydroxydopamine. Naunym
    Schmiedeberg Arch. Pharm. Exp. Path. 261:271-288, 1968.

17. Weller, R.O., Bayliss, O.B., Abdulla, Y.H., et al.: The Electron-
    Histochemical Demonstration of Phosphoglyceride. J. Histochem. Cytochem.
    13:690-692, 1965.

REFERENCES (Continued)

18. Meban, C.: The Inclusion Bodies in Granular Pneumocytes of Hamster Lung: A Combined Cytochemical and Ultrastructural Study. J. Anat. 112:195-206, 1972.

19. Lauweryns, J.M., et al.: Serotonin Producing Neuroepithelial Bodies in Rabbit Respiratory Mucosa. Science 180:410-413, 1973.

20. Cutz, E., Chan, W., Wong, V., and Cohen, D.E.: Endocrine Cells in Rat Fetal Lungs: An Ultrastructural and Histochemical Study. Lab. Invest. 30:458-464, 1974.

21. Nagaishi, C.: Functional Anatomy and Histology of the Lung. London, University Park Press, 1972. (Fig.1)

22. Lentz, T.L.: Cell Fine Structure: An Atlas of Drawings of Whole-Cell Structure. Philadelphia, W.B. Saunders, 1971. (Fig. 2)

23. Weibel, E.R., and Gil, J.: Resp. Physiol. 4:42-57, 1968. (Fig. 3)

TABLE --- LAMELLATED BODY DATA

	No. of Animals	No. of Cells	No. of Lamellated Bodies	Mean Score ± S.E.
Control	7	50	400	4.43± 0.03
6-HODA	5	50	400	3.77± 0.16

p <0.005

B. On-Going Studies

1. Introduction

INTRODUCTION

Controversy still exists over the proper choice of fluids for resuscitation of patients in shock following trauma or sepsis, with particular regard to the incidence of posttraumatic pulmonary insufficiency. Previous work from our laboratory has demonstrated distinct morphologic changes in the pulmonary ultrastructure following hemorrhagic shock.[1,2] We have compared the changes following resuscitation with crystalloid and colloid, and our interest has continued to focus on the differences between the two solutions.

Staub has made significant contributions to the understanding of the fluid and protein composition of lung fluids.[3] We can now classify the two major types of pulmonary edema as that due to increased driving pressure (high pressure edema), and that due to altered integrity of the capillary membrane (permeability edema).[4] An important development has been the recognition that in the normal state, the microvasculature of the lung is not impermeable to proteins. This observation has led to a number of physiologic studies that have examined the effects of hemorrhagic and septic shock on pulmonary capillary membrane permeability.[5,6,7] Although not all in agreement, the general impression has been that protein permeability is increased following sepsis, but not hemorrhage.

Morphologic studies have failed to satisfactorily demonstrate the transfer of plasma proteins across the capillary membrane.[8,9,10,11] Tracers such as ferritin, catalase, hemoglobin, and horseradish peroxidase have been used, and although the endothelial cell has been permeable to many of these macromolecules, it has been shown that some of the substances can alter membrane permeability by themselves. [8,11,12]

Bignon has recently demonstrated autologous albumin in the alveolar capillary membrane and the alveolar lining material using the principles of immunoelectron microscopy.[13,14] The mode of transcapillary transport, however, has still not been conclusively demonstrated.

We have become interested in the techniques of fluorescent microscopy and autoradiography as methods to study the mechanism of protein transport.[15,16] An Evans blue-albumin solution is able to identify the general area of leakage from the microvascular bed, but does not allow any quantitative interpretations. We are also investigating the properties of fluorescein labeled dextrans (FITC-Dextrans) as tracers in morphologic studies of vascular permeability,[17] and plan to use these as markers for use with the electron microscope. Autoradiography with ^{125}I-albumin permits examination of the pinocytotic vesicles and intercellular junctions, and should lead to a better understanding of the transvascular escape of protein. After identifying the normal mechanism, application of the techniques in the hemorrhagic and septic models should demonstrate any altered permeability and help clarify the proper methods of resuscitation in the clinical situation.

REFERENCES

1. Moss, G.S., Das Gupta, T.K., et al. Effect of Hemorrhagic Shock on Pulmonary Interstitial Sodium Distribution in the Primate Lung. Ann. Surg. 177:211, 1973.

2. Moss, G.S., Das Gupta, T.K., et al. The Effect of Saline Solution Resuscitation on Pulmonary Sodium and Water Distribution. Surg. Gyn. and Obstet. 136:934, 1973.

3. Staub, N.C. Pulmonary Edema. Physiol. Rev. 54:678, 1974.

4. Staub, N.C. Pulmonary Edema Due To Increased Microvascular Permeability to Fluid and Protein. Circ. Res. 43:143, 1978.

5. Brigham, K.L., Woolverton, N.C., et al. Increased Sheep Lung Vascular Permeability Caused by Pseudomonas Bacteremia. J. Clin. Invest. 54:792, 1974.

6. Holcroft. J.W., and Trunkey, D.D. Pulmonary Extravasation of Albumin During and After Hemorrhagic Shock in Baboons. J. Surg. Res. 18:91, 1975.

7. Demling, R.H., Selenger, S.L. et al. Effect of Acute Hemorrhagic Shock on Pulmonary Microvascular Fluid Filtration and Protein Permeability in Sheep. Surgery 77:512, 1975.

8. Schneeberger, E.E., and Karnovsky, M.J. The Influence of Intravascular Fluid on the Permeability of Newborn and Adult Mouse Lung to Ultrastructural Protein Tracers. J. Cell Biol. 48:319, 1971.

9. Pietra, G.G., Szidon, J.P., et. al. Hemoglobin as a Tracer in Hemodynamic Pulmonary Edema. Science 166:1643, 1969.

10. Schneeberger, E.E. and Karnoveky, M.J. The Ultrastructural Basis of Alveolar-Capillary Membrane Permeability To Peroxidase Used As A Tracer. J. Cell Bio. 37:781, 1968.

11. Szidon, J.P., Pietra, G.G., and Fishman, A.P. The Alveolar-Capillary Membrane and Pulmonary Edema. New England J. of Med. 286:1200, 1972.

12. Clementi, F. Effect of Horseradish Peroxidase On Mice Lung Capillary Permeability. J. Histochem. Cytochem. 18:887, 1970.

13. Bignon, J., Chakinian, P., et al. Ultrastructural Immunoperoxidase Demonstration of Autologous Albumin in the Alveolar Capillary Membrane and in the Alveolar Lining Material in Normal Rats. J. Cell Biology 64:504, 1975.

14. Bignon, J., Jourand, C., et al. Immunoelectron Microscopic and Immunochemical Demonstrations of Serum Proteins in the Alveolar Lining of the Rat Lung.

15. Nicolaysen, G., and Staub, N.D. Time Course of Albumin Equilibration in Interstitium and Lymph of Normal Mouse Lungs. Micron. Res. 9:29, 1975.

16. Nicolaysen, G., Nicolaysen, A., and Stabu, N.C. A Quantitative Radioautographic Comparison of Albumin Concentration in Different Sized Lymph Vessels in Normal Mouse Lungs. Micron. Res. 10:138, 1975.

17. Olsson, Y., Svensjo, E., et al. Fluorescein Labeled Dextrans As Tracers for Vascular Permeability Studies in the Nervous System. Act. Neuropath. 33:45, 1975.

B. On-Going Studies

   2. Radioautography of ^{125}-Tagged Albumin in the Lung.

RADIOAUTOGRAPHY

OBJECT: To define the pulmonary vascular and extravascular distribution of albumin in the control state and following shock.

METHODS

In order to inject an appropriate amount of isotope for quantifiable radioautography in a small enough fluid volume, a specially prepared, high specific activity 0.25 m Ci/mg ^{125}-labeled albumin (New England Nuclear NEX 076, I-125-BSA bovine serum albumin), with an albumin concentration of 20 mg/ml was used. This is five times the usual specific activity.

A. Fixation

Three anesthetized female mice weighing approximately 25 g. were injected with 0.15 - 0.25 ml of the tracer solution through the tail vein. The tracer solution contained less than 2% free iodide when tested by trichloracetic acid precipitation of the protein.

The lungs of three mice were fixed at thirty, sixty, and ninety minutes after the tracer injection. A few minutes before fixation, each mouse was anesthetized with pentobarbital (100 mg/kg) intraperitoneally. The chest was quickly opened, and a 4% glutaraldehyde solution in a cacodylate buffer was instilled into the pleural cavaties until a minimal fixation was obtained. The lungs were then excised, and samples taken from various areas. The specimens were sliced into blocks about 1mm^3, and immersed in the glutaraldehyde at 4° C. for four hours. Postfixation in 2% osmium tetroxide was then performed for two hours, followed by dehydration in graded acetone solutions and propylene oxide. The blocks were embedded overnight in Araldite.

B. Sectioning

The tissue blocks were cut into 1um thick sections using glass knives in a microtome (LKB-Huxley or a Sorvall JB4). The sections were stained with toluidine blue, and the appropriate blocks selected for further examination. Ultrathin sections were cut, and after being mounted on copper grids, were dip-coated with a nuclear emulsion (Ilford K2) with a 1:1 emulsion water ratio. After appropriate exposure (21-25 days), the emulsion was developed and the sections examined under an RCA EMU 312-4 microscope.

The following figures are the results of these preliminary experiments, and represent a validation of this technique as performed in our laboratory:

Figure 1: Cluster of 125-I-labeled material in the alveolar lumen.

Figure 2: The capillary lumen shows decreased quantities of material.
Most of the tagged material is in the interstitium. We have
no lymphatic lumen visualized in this micrograph.

Figure 3: Shows the tagged material in a Type II granular pneumocyte.

C. Shock

The next phase of the project is designed to further characterize the time course of the passage of the albumin from the vascular to the extravascular space, and to delineate the mode of transport. Different time intervals following injection, and various methods of fixation will be used. When this has been completed, the model will be used to study the effects of both hemorrhagic and septic shock on the handling of albumin by the lung.

I. Hemorrhage

Hemorrhagic shock will be induced by judicious bleeding from a tail vein. The level of shock will be monitored by a small pneumatic cuff on the tail connected to an electromagnetic flowmeter, a technique shown to be as accurate in the mouse as intraarterial monitoring.

II. Sepsis

The septic model involves ligation and puncture of the cecum, with subsequent peritonitis. Further experience will help determine the proper timing of the fixation process after the septic event.

LEGENDS

FIG. 1   A tracing of the densitometric scan of a gel electrophoresis
          strip spotted with baboon serum albumin (upper panel) and
          human serum albumin (lower panel).

FIG. 2a   Low power view of normal baboon lung showing localization of
          sodium pyroantimony. The capillary contains a number of red
          blood cells and a portion of a platelet (P). The interstitium
          shows regular banding of collagen (CO) with discrete crystalline
          localization of sodium pyroantimony (NA). A portion of a
          granular pneumocyte with dense lamillate bodies within the cyto-
          plasm can be seen in the upper right of the micrograph. x 3,200.

FIG. 2b   High power view of normal lung. The precise locatization of
          the sodiumpyroantimony (NA) in and around the collagen (CO)
          fibers within the interstitium (IS) can be seen. Note the
          distribution in two planes: in transverse section sodium can
          be found along the outer rims of the individual collagen fibers;
          in longitudinal sections the sodium pyroantimony deposits have
          aggregated in geometric precision along the silhouette of the
          fibers. x 29,500

FIG. 3a   Lower power view of a baboon lung in hemorrhagic shock, showing
          distribution of sodium pyroantimony. Note the characteristic
          interstitial edema with smudging of sodium pyroantimony along
          the collagen fibers (CO). It appears that collagen (CO) has a
          spongelike property for the sodium-rich edema fluid. x 12,000

FIG. 3b.   Higher power view of a similar field as in Fig. 3a. The

smudging and disruption of the interstitial space are

magnified. x 20,000

FIG. 4a.   Baboon lung resuscitated with Ringer's lactate. The

interstitial space is relatively normal. Collagen fibers do

not appear disrupted. Small areas of edema-rich sumdges are

still seen. x 20,000

FIG. 4b.   Another area from the same animal (Fig. 4a) showing the

collagen. Note the distribution of sodium appears similar to

that in the control (Figs. 2a and 2b). Although minor amounts

of edema and smudge still persist, the general appearance of

the pulmonary interstitium is similar to the control baboon

lungs. x 27,000

FIG. 5a.   Baboon lung after resuscitation with human serum albumin. Note

the marked interstitial edema, dispersal of collagen fibers,

(CO) and patchy areas of smudging of sodium pyroantimony.

x 28,500

FIG. 5b.   Another area of the pulmonary interstitium from the

same lung as in Fig. 5a. The pattern of architectural dis-

integration of the interstitium is similar to Fig. 3b. The

distribution of sodium-rich edema smudges are more spectacular.

Furthermore, disorganization of collagen periodicity is apparent

x 28,500.

FIG. 6a. Lung tissue after resuscitation with baboon albumin. The
interstitial space shows edema, disintegration of periodicity
of collagen, and edema-rich smudges. A granular pheumocyte
can be seen on the upper right of the micrograph. x 19,500

FIG. 6b. A higher view of the interstitium from the same baboon
(Fig. 6a). The interstitial edema, collagen disruption, and
nongeometric localization of sodium can be easily appreciated.
x 28,500

TABLE 1. <u>Infusion Volumes (cc/kg)</u>

<u>Group</u>	<u>Mean</u>	<u>S.D.</u>	<u>N</u>
Ringer lactate	148	17	3
Human serum albumin	63	11	3
Baboon Serum albumin	65	14	3

References

1. Brigham, K.L., and Staub, N.C.: Lung Interstitial Protein: Studies of Lung Lymph. Proceedings of the Workshop on Albumin. Sponsored by: The Division of Blood and Blood Products, Bureau of Biologics and The Division of Blood Diseases and Resources, National Heart and Lung Institute. Edited by: James T. Sgouris and Anthony Rene, February, 12-13, 1975.

2. Cohn, E.J., Strong, L.E., Hughes, W.L., Mulford, D.J., Ashworth, J.W., Melin, M., Taylor, H.L.: Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lipoprotein Components of Biological Tissues and Fluids. J. Amer. Chem. Soc. 68:459, 1946.

3. Das Gupta, T.K., Moss, G.S., and Newson, B.: Localization of Sodium Ion in the Normal Primate Lung. An Ultrastructural Study. Acta Anat. 80:426, 1971.

4. Lowe, R.J., Moss, G. S., Jilek, J. Levine, H.D.: Crystalloid Vs Colloid in the Etiology of Pulmonary Failure After Trauma - A Randomized Trial in Man. Surg. 81:676, 1977.

5. Moss, G.S., Das Gupta, T.K., Newson, B., and Nyhus, L.M.: Morphologic Changes in the Primate Lung Following Hemorrhagic Shock. Surg. Gynec. Obstet., 143:3, 1972.

6. Robin, E.D., Carey, L.C., Grenvik, A., et al.: Capillary Leak Syndrome with Pulmonary Edema. Arch. Intern. Med., 130:66, 1972.

7. Siegel, D.C., Moss, G.S., Cochin, A., and Das Gupta, T.K.: Pulmonary Changes Following Treatment of Hemorrhagic Shock. Surg. Forum, 21:17, 1970.

8. Starling, E.: On the Absorption of Fluid from the Connective Tissue Spaces. J. Physiol. 19:3;2, 1895.

9. Virgilio, R.W., Smith, D.W., Rice, C.L., Hobelmann, C.L., Zarins, C.K., James, D.R., and Peters, R.M.: Effect of Colloid Osmotic Pressure and Pulmonary Capillary Wedge Pressure on Intrapulmonary Shunt. Surg. Forum, 27:168, 1976.

10. Zarins, C.K., Rice, C.L., Smith, D.E., John, D.A., Cammack, B.F., Peters, R.M., and Virgilio, R.W.: Role of Lymphatics in Preventing Hypooncotic Pulmonary Edema. Surg. Forum. 27:257, 1976.

FIGURE 1:

FIGURE 2:

FIGURE 3:

MOSS ET AL.
"Radioautography of 125-I Albumin..."

B. On-Going Studies

    3. Fluorescent Micrography in the Lung.

OBJECTIVE: To obtain more direct information regarding the permeability
of the lung microvasculature to plasma proteins, using fluorescent labeled
tracer substances.

METHODS

A. Evans Blue Albumin:

I. Fixation

0.6g of Evans blue (J.T. Baker Co.) and 4g of bovine serum
albumin (B.S.A.) (Sigma) are dissolved in 100 ml 0.9% Na Cl. 0.1 ml of
the labeled albumin is injected into the tail vein of unanesthetized mice
(body weight 20-25g, Charles River, SPF). At selected intervals between
ten minutes and five hours after injection, each animal is anesthetized
with pentobarbital (100 mg/kg) intraperitoneally. A tracheostomy is performed,
the chest is opened, and the lungs are inflated to a constant transpulmonary
pressure of 10 cm H_2O. The inflated lungs are frozen in the open thorax
using liquid propane cooled to -180° C. in liquid nitrogen. The excised
lungs are freeze-dried at -35° C. for 24 hours, and suitable tissue blocks
are vacuum infiltrated and embedded in paraffin.

II. Sectioning and Microscopy

Sections of 10μm thickness are cut on a sliding microtome, and after
removal of the paraffin by heat, are mounted in nonfluorescing oil. The
sections will be examined using a fluorescence microscope with a mercury
lamp, using dark field illumination, a BG 12 excitation filter, and a
barrier filter with cut-off at 530 mn.

Preliminary evaluation will establish suitable criteria for recording
the time course and distribution of the fluorescence. Sections will then
be presented in random, unidentified fashion to two different investigators,

and the appearance and distribution of the fluorescence will be graded on a 0 - 3+ scale. The results will help determine the areas of the lung architecture to be examined more carefully using techniques suitable for electron microscopy.

III. Shock

When the technique has been satisfactorily performed in the control animals, the model will be used in both hemorrhagic and septic shock.

A. Hemorrhage

Hemorrhagic shock will be induced by judicious bleeding from a tail vein. The level of shock will be monitored by a small pneumatic cuff on the tail connected to an electromagnetic flowmeter, a technique shown to be as accurate in the mouse as intraarterial monitoring.

B. Sepsis

The septic model involves ligation and puncture of the cecum, with subsequent peritonitis. Further experience will help determine the proper timing of the fixation process after the septic event.

B. Fluorescein Labeled Dextrans

I. Technique

Fluorescein isothiocyanate labeled dextrans (FITC-Dextran) have previously been used for direct observations of vascular permeability in living animals, and there is recent evidence to suggest their value as electron microscopical tracers. The advantage of these molecules as tracers is their wide range of molecular sizes. Efforts are currently underway to obtain these labeled compounds for use as tracers in the baboon.

II. Shock

Following the initial experience with two control animals in the normal state, the influence of both hemorrhagic and septic shock will be examined.

A. Hemorrhage

Hemorrhagic shock will be induced in four baboons by a standard model of bleeding from the femoral artery into a sterile plastic bag to lower the mean arterial pressure to 60 mm Hg for one hour, and to 40 mm Hg for the second hour. The animals will then be sacrificed and the lungs examined under the electron microscope after standard tissue processing.

B. Sepsis

Sepsis will be induced in four baboons by infusion of live E. Coli organisms, Dunwald strain B15, (obtained from Dr. L.B. Hinshaw, V.A. Hospital, Oklahoma City). Lyophilized samples are reconstituted, cultured, and recovered for injection. A standard suspension is obtained using optical density read at 550 nm, and subsequent colony counts determine the bacterial concentration. After early dissatisfaction with bolus injection,

efforts are now being focused on a four hour infusion of the bacterial
suspension. Hemodynamic and respiratory parameters are being monitored
in an attempt to establish a dose-response curve and thus a reproducible
model. Sepsis will then be induced for the four hours, followed by
sacrifice of the animals and examination of the lungs under the electron
microscope.

C. Budget

MICHAEL REESE HOSPITAL AND MEDICAL CENTER

Treatment of Hemorrhagic Shock
Saline vs. Colloid

Principal Investigator - Gerald S. Moss, M.D.
Contract No.   N00014-75-C-1086
Task No. NR-105-539

Budget: 12/1/78 - 11/30/79

1. Salaries

    a. Gerald S. Moss, M.D. (Principal Investigator, 20% time effort $ -0-
    b. Biochemist (100% time and effort) $ 10,260.00
    c. Fringe Benefits (FICA 6.13%) $ 629.00
    d. Indirect Cost (75% of salary and wages) $ 7,695.00
    $ 18,584.00

2. Supplies and Reagents
    a. Electronmicroscopy Stains $ 800.00
    b. Photographic Paper and Emulsions $ 285.00
    c. I.V. Fluids and Tubing $ 350.00
    d. 125 I-Albumin @ $250.00 for 5mCi x 9 doses $ 2,250.00
    $ 3,685.00

3. Experimental Animals
    a. 10 Baboons @ $550.00 $ 5,500.00
    b. Freight and handling on Baboons @ $88.00 $ 880.00
    c. Per diem $1.80 for each animal for 30 days $ 540.00
    d. 100 Mice at $0.71 each $ 71.00
    e. Freight on mice @ $25.00 each for 3 shipments $ 75.00
    f. Per diem $.0475 for each animal 10 days $ 47.50
    $ 7,113.50

    TOTAL $ 29,382.50

Budget Justification:

1. The requested salary provides a 8.0% increase over last year's contract salary level. We feel that the salary level is competitive for the Chicago, Illinois job market.

2. Supplies and Reagents classification reflects the total supplies needed for the current research level.

3. Experimental Animals: We continue to believe that baboons and mice are the appropriate animals for these studies for both physiological and economic reasons.